

The process of hemostasis occurs in three phases: the vascular platelet phase, which assures primary hemostasis; activation of the coagulation cascade, which assures formation

of the clot; and activation of a series of control mechanisms, which stop propagation of the clot and limit activation of the coagulation cascade to the region of endothelial rupture.

Tests of the Vascular Platelet Phase of Hemostasis

Bleeding Time

Definition

When a small slit is made in the skin, the hemostatic mechanisms necessary for coagulation are activated. Without the aid of external pressure, bleeding usually stops within 7 to 9 minutes.

Technique

The test is performed using a disposable template that produces a uniform incision. The incision, either horizontal or vertical, is placed on the lateral aspect of the forearm, about 5 cm below the antecubital fossa, after a blood pressure cuff has been inflated to approximately 40 mm Hg. Blood may be absorbed off the skin, but care must be taken to avoid pressure. The time is measured from the moment of incision to the moment bleeding stops. The time may vary based on the commercial template used, the direction of the incision, and the location on the arm. Each institution must establish its own upper limits of normal.

Basic Science

The vascular platelet phase of hemostasis consists of a primary vasoconstriction that serves to decrease blood flow,

followed by adherence of platelets to the ruptured endothelium (adhesion) and each other (aggregation). This platelet aggregate, called the *platelet plug*, stops the bleeding and forms a matrix for the clot. The bleeding time is an excellent screening test for the vascular platelet phase of hemostasis. It depends on an intact vasospastic response in a small vessel and an adequate number of functionally active platelets.

Clinical Significance

Patients with abnormalities of the vascular platelet phase of hemostasis present with purpura (petechiae and ecchymoses) and spontaneous bruising. They may have mucosal bleeding and fundus hemorrhages. Commonly, the problem is either thrombocytopenia, easily evaluated by a platelet count, or abnormal platelet function, which can be diagnosed with platelet function studies. The most common acquired platelet function abnormalities are drug induced (aspirin and the nonsteroidal anti-inflammatory agents) and uremia. The most common hereditary abnormality is von Willebrand's disease.

Tests of the Coagulation Cascade

These in vitro tests—the activated partial thromboplastin time (aPTT), prothrombin time (PT), and thrombin time (TT)—measure the time elapsed from activation of the coagulation cascade (Figure 157.1) at different points to the generation of fibrin.

Activated Partial Thromboplastin Time

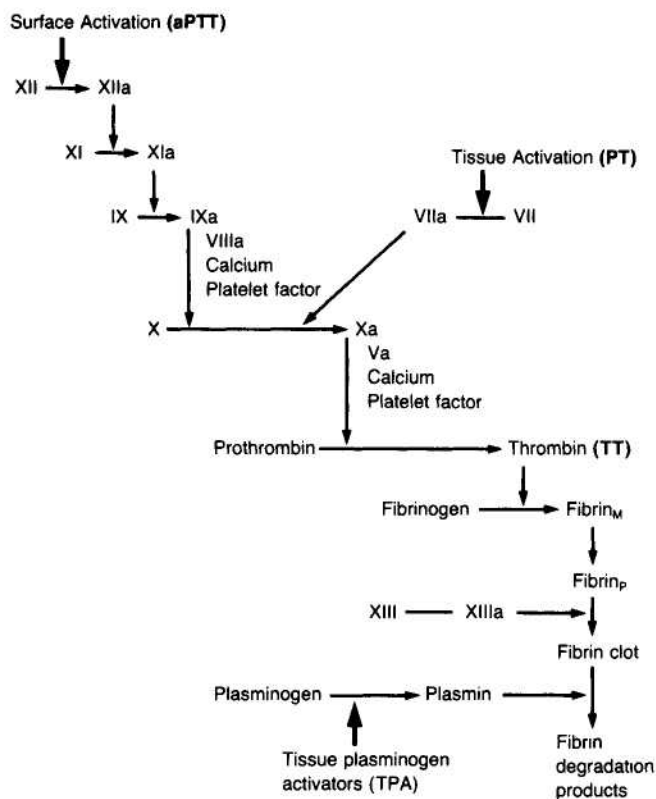
Definition

The aPTT measures the time necessary to generate fibrin from initiation of the intrinsic pathway (Figure 157.1). Activation of factor XII is accomplished with an external agent

(e.g., kaolin) capable of activating factor XII without activating factor VII. Since platelet factors are necessary for the cascade to function normally, the test is performed in the presence of a phospholipid emulsion that takes the place of these factors. The classic partial thromboplastin time depends on contact with a glass tube for activation. Since this is considered a difficult variable to control, the “activated” test uses an external source of activation.

Technique

Citrated plasma, an activating agent, and phospholipid are added together and incubated at 37°C. Calcium is added,

**Figure 157.1**

The coagulation cascade. Bold type indicates the starting point of the coagulation test noted.

and the time necessary for the clumping of kaolin is measured. The normal time is usually reported as less than 30 to 35 seconds depending on the technique used. In fact, there is a normal range of about 10 seconds (e.g., 25 to 35), and decreased values ("short") may also be abnormal.

Basic Science

This test is abnormal in the presence of reduced quantities of factors XII, IX, XI, VIII, X, V, prothrombin, and fibrinogen (all integral parts of the "intrinsic" and "common" pathway). It is usually prolonged if a patient has less than approximately 30% normal activity. It can also be abnormal in the presence of a circulating inhibitor to any of the intrinsic pathway factors. The differentiation of inhibitors from factor depletion is important and can best be accomplished by a *mixing study* in which patient and normal plasma are combined in a 1:1 ratio and the test is repeated on the mixed sample. If the abnormal value is corrected completely, the problem is factor deficiency. If the result does not change or the abnormality is corrected only partially, an inhibitor is present. This difference stems from the above mentioned fact that the aPTT will be normal in the presence of 50% normal activity.

Clinical Significance

The aPTT is a good screening test for inherited or acquired *factor deficiencies*. Inherited disorders including classic hemophilia A (factor VIII deficiency) and hemophilia B (factor

IX deficiency, or Christmas disease) are well-known diseases in which the aPTT is prolonged. Other intrinsic and common pathway factors may also be congenitally absent. These conditions are rare but have been described for all factors. A number of kindreds with abnormalities of factor XII activation have been described. They are usually associated with a prolonged aPTT without clinical signs of bleeding. Acquired factor deficiency is common. Vitamin K deficiency, liver dysfunction, and iatrogenic anticoagulation with warfarin are most common. Factor depletion may also occur in the setting of disseminated intravascular coagulation (DIC), prolonged bleeding, and massive transfusion.

A prolonged aPTT that cannot be completely normalized with the addition of normal plasma can be explained only by the presence of a circulating *inhibitor of coagulation*. The presence of these inhibitors is almost always acquired, and their exact nature is not always apparent. From a clinical point of view, the most common inhibitors should be considered *antithrombins*. These compounds inhibit the activity of thrombin on the conversion of fibrinogen to fibrin (Figure 157.1). The two most common inhibitors are heparin, which acts through the naturally occurring protein antithrombin III (AT III), and fibrin degradation products (FDP), formed by the action of plasmin on the fibrin clot and usually present in elevated concentrations in DIC and primary fibrinolysis.

Other inhibitors appear to be antibodies. The easiest to understand is the antibody against factor VIII in patients with hemophilia A treated with factor VIII concentrate. Inhibitors against other factors have been described with a variety of diseases that follow a variable course. When characterized, they have been immunoglobulins.

A particular problem may be seen in patients suffering from systemic lupus erythematosus. These patients may present with a prolonged aPTT without evidence of bleeding. Some present with thrombosis. The abnormality cannot be corrected with normal plasma and has been referred to as the "lupus anticoagulant." This phenomenon does not represent an *in vivo* problem with the coagulation cascade. Rather, it is a laboratory abnormality caused by the presence of a serum constituent that interferes with the *in vitro* partial thromboplastin test.

Occasionally the reported value of the aPTT will be lower than normal. This "shortened" time may reflect the presence of increased levels of activated factors in context of a "hypercoagulable state." It is seen in some patients in the early stages of DIC but should not be considered diagnostic for that entity.

Prothrombin Time

Definition

The PT measures the time necessary to generate fibrin after activation of factor VII. It measures the integrity of the "extrinsic" and "common" pathways (factors VII, V, X, prothrombin, and fibrinogen).

Technique

Citrated plasma and an activating agent (usually thromboplastin extracted from animal brain) are incubated at 37°C. The plasma is recalcified and the time is measured until

fibrin filaments are observed. Each laboratory has its own normal value, usually between 12 and 15 seconds.

Basic Science

As with the interpretation of a prolonged aPTT, a prolonged PT may reflect either factor deficiency or a circulating inhibitor of coagulation. The distinction is made by repeating the test after a 1:1 mix with normal plasma.

The test is more sensitive than the aPTT for deficient levels of factors, and a relatively small drop in factor VII levels may prolong the PT.

Clinical Significance

Inherited deficiency of factor VII is a rare bleeding disorder characterized by a prolonged PT and a normal aPTT. The PT completely corrects when mixed with normal plasma. Acquired deficiencies are usually related to liver disease, warfarin therapy, or depletion secondary to consumptive coagulopathy, severe bleeding, or massive transfusion.

Circulating inhibitors are most often directed at factor X or thrombin. Most common are heparin or products of fibrinolysis. In their presence the prolonged PT cannot be completely corrected to normal in a 1:1 mixing study.

Thrombin Time

Definition

This test measures the time necessary to drive the reaction of fibrinogen to fibrin in the presence of thrombin. It measures the integrity of this reaction and isolates an abnormality to either a decrease in normal fibrinogen or an inhibitor to its activation.

Technique

Citrated plasma is incubated at 37°C and thrombin is added to the solution. Time is measured from the addition of thrombin to the generation of fibrin filaments. Calcium is unnecessary.

Basic Science

Abnormalities can be explained in one of three ways: deficient fibrinogen (< 100 mg/dl), abnormal fibrinogen, or an inhibitor to the reaction. As with other tests of the coagulation cascade, if a 1:1 mixing study normalizes the prolonged time, one is dealing with factor deficiency. As it pertains to fibrinogen, however, one must distinguish a decrease in normal fibrinogen from the production of an abnormal fibrinogen (dysfibrinogenemia).

Clinical Significance

Acquired deficiency of fibrinogen is usually due to a consumptive coagulopathy or, less often, severe liver disease. Hereditary deficiencies exist, but with variable clinical presentations. Afibrinogenemia is an often fatal childhood condition.

Abnormal fibrinogen (dysfibrinogenemia) can be acquired or inherited. The acquired form is usually found in association with severe liver disease, but has been reported in other diseases. The congenital form is rare, usually autosomal dominant. A discordance between immunologic and physiologic measurements of fibrinogen is the key to diagnosis.

The most common acquired inhibitors of this reaction are heparin and fibrin degradation products (FDP). The effect of heparin can be eliminated by catalyzing the reaction with reptilase, which, unlike thrombin, is insensitive to heparin. FDP are commonly seen in consumptive coagulopathies and primary fibrinolytic states.

— Tests of Fibrinolysis and the Mechanisms That Control Hemostasis —

As described in Figure 157.1, the coagulation cascade is an efficient system for generating fibrin. Equally important is the system that limits this process, neutralizes activated factors, stops propagation of the clot, and keeps the process of coagulation confined to the area of endothelial rupture.

Fibrinolysis is accomplished by the action of plasmin on fibrin polymer. Plasmin is generated from plasminogen (produced by the liver) by the action of plasminogen activators. These compounds are present in endothelial cells, and the reaction is accelerated in the presence of fibrin. Thus the generation of fibrin appears to turn on the fibrinolytic process and localize the formation of the fibrin gel.

Fibrin Degradation Products

Definition

As a marker of fibrinolysis, fibrin degradation products (FDP), also known as fibrin split products (FSP), can be

quantified by a test based on latex agglutination. The test uses antibodies to FSP which are measured using serial dilutions.

Technique

Serum is prepared in a series of dilutions (e.g., $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{16}$). Latex particles onto which have been absorbed antibodies to FSP are added. If agglutination is seen, the test is positive at that dilution. The test is reported as the most dilute sample that agglutinates. The normal value is less than $\frac{1}{4}$ to $\frac{1}{16}$.

Basic Science

Although well standardized and easy to perform, the FSP value may be difficult to interpret. The action of thrombin on fibrinogen is to cleave the protein and produce smaller

compounds called fibrinopeptides, plus the fibrin monomer. This monomer polymerizes to form the fibrin gel. The gel is stabilized by the action of factor XIII, activated by thrombin.

The action of plasmin is to cleave both fibrinogen and fibrin. Its action is localized by its activation at the site of endothelial rupture, and the tight association of plasminogen and fibrin. The activity on fibrinogen forms small fragments, D and E. The action on fibrin polymer is to form larger fragments. These fragments are anticoagulants formed at the site of coagulation and serve to inhibit the action of thrombin on fibrinogen to form fibrin. Both are also measured by the technique described above.

Clinical Significance

Increased FDP is the laboratory expression of increased fibrinolysis. This may be part of a local problem of fibrin generation such as brain trauma, chronic bleeding, vascular thrombosis, prostate surgery, uterine disorders, or malignancy, or a systemic process, usually DIC. Patients with severe liver disease can have increased fibrinolysis on the basis of poor clearance of circulating plasminogen activators.

Other Control Mechanisms

At least two other systems are important in controlling the hemostatic process. *Antithrombin III* (AT III) is a protein produced by the liver. It binds thrombin and activated factor X irreversibly. This interaction is accelerated by heparin, which may be associated with ruptured endothelial cells. AT III can be quantified, and is congenitally absent in some patients prone to thrombosis. The technical aspects of this measurement are beyond the scope of this chapter.

A similar protein (protein C) appears to inactivate factors V and VIII in a reaction catalyzed by thrombin, localized

to the area of endothelial rupture. It is a vitamin K dependent protein produced by the liver. Its measurement is still considered a research tool.

Both of these proteins are locally activated at the site of endothelial rupture. They serve to prevent the escape into the circulation of activated factors and limit propagation of the clot.

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